SHORT COMMUNICATION

A Lack of Nuclear DNA Content Variability Among Wheat Near Isolines Differing in Aluminium Response

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Nucleotypic variation has been speculated to play a role in the adaptation of crop species to environmental stress. The objective of this study was to determine if nuclear DNA content variability was associated with aluminium (Al) tolerance in wheat. Six wheat (*Triticum aestivum* L.) near isolines (differing in Al response), two recurrent parents (Al sensitive), and one donor parent (Al tolerant) were all analysed for nuclear DNA content using flow cytometry. A 1·7% variation in nuclear DNA content was observed among the nine wheat lines. No association between Al response and nuclear DNA content was observed. All of the wheat near isolines had a nuclear DNA content similar to their recurrent parent. The wheat genome appears to be stable with no unusual inheritance of nuclear DNA content observed. Flow cytometric analysis proved to be sensitive enough to detect nuclear DNA content variability at the level of 0·5% variation among wheat lines. © 1999 Annals of Botany Company

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INTRODUCTION

Since the early 1960s intraspecific nuclear DNA content variation has been well documented in angiosperm species (Bennett, 1985). Since the discovery of intraspecific variation in nuclear DNA content, the question of its significance has been debated. In 1974, Flavell *et al.* observed that much of the variation in DNA was in the repetitive, presumably non-transcribed regions of the plant genome. In fact it has been estimated that 90–99% of nuclear DNA within the average angiosperm is non-genic DNA (Flavell, 1980). Several reports demonstrated that important cellular characteristics such as chromosome size, cell size and mitotic and meiotic cycle times were associated with this nuclear DNA content variation (Van't Hof and Sparrow, 1963; Bennett, 1971; Bennett, Gustafson and Smith, 1977). After these initial reports, whole organismal characters were found to be associated with nuclear DNA content variation. These characters included minimum generation time (Bennett, 1972). Bennett (1973) used the term nucleotype to describe the effect of the physical aspects of nuclear DNA mass on the phenotype of an organism.

Rayburn *et al.* (1985) noticed nucleotypic effects on maize (*Zea mays* L.), a major agronomic crop species. Characteristics such as latitude and altitude of adaptation, flowering time, growing degree days, and chloroplast number per guard cell have all been shown to be associated with nuclear DNA content variation (Rayburn *et al.*, 1985; Rayburn, 1990; Bullock and Rayburn, 1991; Ho and Rayburn, 1991). Biradar, Bullock and Rayburn (1994) noted that growth

and yield parameters were associated with DNA content variation in maize as well. McMurphy and Rayburn (1991) noted that the more intensive the breeding programme involved in the development of a cultivar, the lower the association between DNA variability and agronomic characteristics.

While initially this seems to be of little consequence, it must be realized that nuclear DNA content variation in crop species (or their primitive relatives) is being lost due to the narrow number of nucleotypes in the breeding pool. This nucleotypic variation could play an important role in the adaptation of crop species to changing environmental conditions. It has been documented that the eukaryotic genome can adapt to various stresses by DNA amplification in both animal and plant cells (Schimke *et al.*, 1978; Shah *et al.*, 1986). Gene amplification is speculated to be a contributing mechanism to the fluidity of the eukaryotic genome (Bachmann, 1993). In addition, if a breeding scheme induced unwanted DNA variation into a plant selection, this variability could be counterproductive to the breeding programme. Such unexpected DNA content variation has been observed during the production of F_1 hybrid maize (Rayburn *et al.*, 1993; Rayburn, Bashir and Biradar, 1997).

The purpose of this study was to determine whether the development of near isolines (an important plant breeding scheme) induced DNA variability. This variability could result from either of two sources. DNA content variability could have changed as a result of the selection for aluminium tolerance or as a result of the breeding scheme used to incorporate the aluminium tolerance into specific lines.

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MATERIALS AND METHODS

Nine wheat lines were used in this study (Table 1). Three cultivars, Atlas 66 (aluminium tolerant), Chisholm and Century (both aluminium susceptible) and six near isolines were examined. The near isolines differ in their aluminium response, but are noted to have a 97% genetic similarity among themselves and 91% genetic similarity with their recurrent parent (Carver et al., 1993). Seeds were provided by Dr B. F. Carver (Oklahoma State University, USA). The tetraploid wheat, Langdon durum, was obtained from the Wheat Genetics Resource Center, Manhattan, KS, USA.

Seeds for all nine lines and the standard tetraploid wheat were planted in Terra-Lite Metro mix 2000 medium (Hummert Seed Co. St Louis, MO, USA). (Mention of trade names or commercial products is solely to provide specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.) All lines were planted on each of 12 d. These days constituted twelve replications of the nine lines and the standard. Exactly 3 weeks after each planting, one plant from each hexaploid wheat line was harvested (twelve harvests in all). A 4·5 cm portion of the stem was chopped into 1 mm discs and was placed into the extraction buffer of Rayburn et al. (1989). A 2·0 cm portion of the tetraploid wheat internal standard was also placed into each sample. The tissue was homogenized for 25 s and then passed through a series of 250, 53 and 20 µm nylon meshes. Samples were centrifuged at 1100 rpm for 15 min. The supernatant was aspirated and the pellet was resuspended in 1 ml of ice cold 70% ethanol for 10 min. The samples were again centrifuged at 1100 rpm for 10 min. The ethanol was aspirated and the pellet washed in 1 ml extraction buffer. After the final centrifugation of

10 min, the nuclei were stained with propidium iodide (PI) following the method of Rayburn, Auger and McMurphy (1992). Samples were coded for analysis. After staining and storing in the dark for at least 1 h, the nuclei were analysed on a Coulter Epics-752 flow cytometric-cell sorter (Coulter Electronics, Hialeah, FL, USA). The excitation wave length was provided by a 5 W argon-ion laser. The excitation wavelength for PI was 488 nm. A minimum of 5000 nuclei per sample were analysed. The DNA content of the nine wheat lines was then estimated compared to the standard.

The experiment was run as a randomized complete block design with 12 blocks (replications) and nine lines. The lines were not decoded until the statistical analysis. Tests using LSD and contrast were carried out to detect differences between lines, between Al response and between recurrent parentage.

After statistical analysis, four of the near isolines were chosen for reanalysis. PI561727 and PI561725 were selected from the Century near isolines and PI561726 and PI561722 were selected from the Chisholm near isolines. On each of 7 d, the four lines were planted along with tetraploid wheat standard. After 3 weeks, one plant per line was harvested and analysed as described previously. Statistical analysis was performed as described above.

RESULTS

There was no overlap between the tetraploid and hexaploid G1 and G2 peaks (Fig. 1). Estimates of relative DNA content of the hexaploid peak could be made without bias. The hexaploid wheat was observed to have about 36% more DNA than the tetraploid wheat. The tetraploid wheat was defined as having a nuclear DNA amount of 100 arbitrary units (AU). The hexaploid wheat DNA amounts ranged from 135·7 to 138·0 AU (Table 1). An overall variation of 1·7% was noted in nuclear DNA content between the lines.

Analysis of variance to test nuclear DNA content differences showed a significant difference among days and lines. The day effect was significant at $Pr > F = 0.0018$. This was somewhat surprising since an internal standard was used. Upon further analysis of the data, it was noted that the majority of measurements on day 3 were lower than the rest of the days. Day 3 seemed responsible for the significant day effect. Since the model used for the statistical analysis takes into account the day effect, the significant day effect did not interfere with the analysis of the other effects analysed. It should be noted, however, that if the model used in the analysis did not take into account the day effect, further analysis could have been misleading.

Upon analysis of the DNA content differences among lines, a significant difference was observed with $Pr > F = 0.0001$. The LSD test showed a critical value of 0·7 AU at $P = 0.05$ (Table 1) indicating that a difference of 0·5% could be detected. Two of the parental lines, Chisholm (aluminium sensitive) and Atlas (aluminium tolerant) had approximately the same DNA content (135·8 AU and 136·0 AU, respectively). The other aluminium sensitive recurrent parent, Century, had significantly more nuclear DNA (137·5 AU; Table 1).

TABLE 1. LSD analysis on nuclear DNA content of the wheat lines

Identification Number	Background	Aluminium response	n	Mean (arbitrary units)*	T grouping†
Experiment 1					
PI561727	Century	Sensitive	12	138·0	A
PI561724	Century	Tolerant	12	137·7	A
PI561725	Century	Tolerant	12	137·6	A
Century	—	Sensitive	12	137·5	A
PI561726	Chisholm	Sensitive	12	136·2	B
PI561722	Chisholm	Tolerant	12	136·2	B
Atlas 66	—	Tolerant	12	136·0	B
Chisholm	—	Sensitive	12	135·8	B
PI561723	Chisholm	Tolerant	12	135·7	B
LSD = 0·7					
Experiment 2					
PI561727	Century	Sensitive	7	137·9	A
PI561725	Century	Tolerant	7	137·7	A
PI561726	Chisholm	Sensitive	7	136·0	B
PI561722	Chisholm	Tolerant	7	135·8	B
LSD = 0·4					

* Relative to the internal standard, Langdon wheat, defined as having 100 AU.

† Means with the same letter are not significantly different at $P = 0.05$.
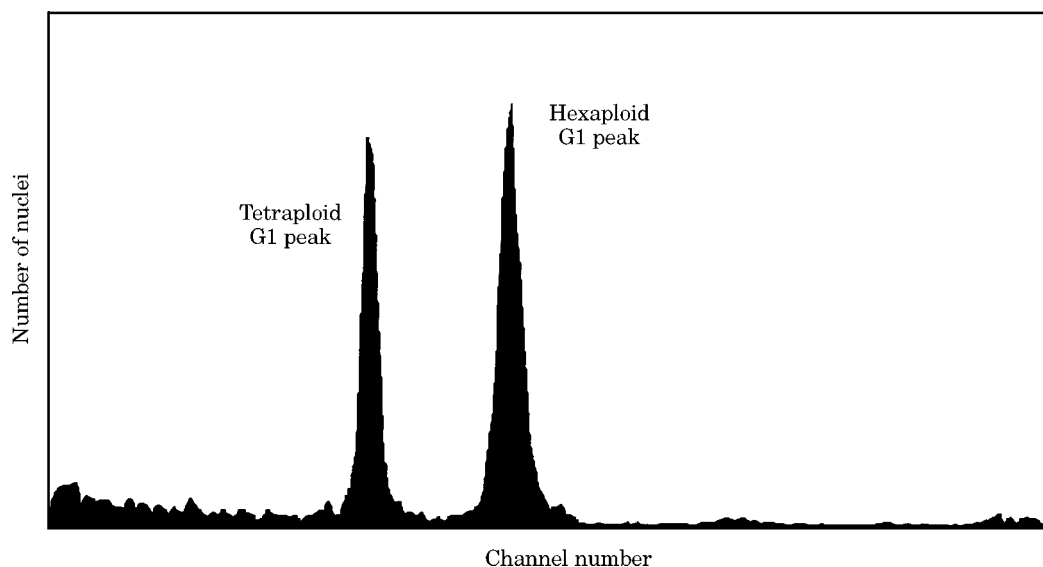
Fig. 1. Flow histogram of hexaploid wheat with internal tetraploid wheat standard.

The mean nuclear DNA contents of the Chisholm near isolines were not significantly different from each other. The mean nuclear DNA contents of the Century lines were also not significant at $P = 0.05$. Atlas had a mean nuclear DNA content amount significantly different from the Century near isolines but not the Chisholm near isolines. Century and its related near isolines were observed to have significantly more nuclear DNA than Chisholm and its related near isolines. Upon contrast analysis, a significant difference with a $Pr > F = 0.0001$ was observed between the Chisholm near isolines and the Century near isolines. Neither the LSD test nor contrast test indicated any significant difference between the aluminium tolerant and aluminium sensitive cultivars or near isolines.

Re-analysis of the four near isolines gave the same results. A significant difference was observed with respect to nuclear DNA content. The nuclear DNA content of the Century lines was significantly different to that of the Chisholm near isolines (Table 1). No significant difference with respect to aluminum tolerance was observed ($P = 0.80$).

DISCUSSION

The small nuclear DNA content variation observed in the wheat lines was not unexpected. The relative consistency of reported genome sizes of wheat over the years and from many laboratories suggests that genome size variation, not associated with aneuploidy, is quite low in wheat (Bennett and Leitch, 1997). In fact, due to its relatively stable genome size, wheat has been suggested as a calibration standard to be used between laboratories studying DNA content variation in plants. Such low variability and consistency of results gave an LSD critical value of 0.7 AU. Therefore lines with approximately a 0.5% difference in nuclear DNA content between them would be significantly different at the level tested. Low variability within plant species is not uncommon. Several studies in different species have noted a lower amount of DNA variability than that found in maize

(for a complete review of this topic, see Bennett and Leitch, 1995).

The purpose behind development of the isolines was to move the aluminium tolerance from the soft red wheat Atlas into the hard red wheat background of Century and Chisholm. The near isolines are reported to be 97% similar within each background and 91% genetically similar with their recurrent parent (Carver *et al.*, 1993). The milling and flour quality of each background was also recovered (Carver *et al.*, 1993). The genome size data is in agreement with the genetic similarity estimates.

All three near isolines within each background had statistically similar genome sizes. In addition, each set of near isolines had statistically similar genome sizes to that of their respective recurrent parent. Century was observed to have 1.3% more DNA than Chisholm. This difference exceeded the LSD critical value and was statistically significant. The mean DNA content of Atlas was 136.0 AU which was significantly lower than the 137.5 AU found in Century but not significantly different than the 135.8 AU found in Chisholm. All of the near isolines of Century had genome sizes statistically similar to Century. This indicates that the number of backcrosses that were used to develop these near isolines were sufficient to recover the nuclear DNA content of the recurrent parent. Indications are that no unusual phenomena are occurring that result in unexpected DNA variation. These results support the hypothesis of a stable genome in wheat. In maize, large genome size variation and the unusual DNA variation that occurs is indicative of a more unstable genome (Rayburn *et al.*, 1985; Porter and Rayburn, 1990; Rayburn, 1990; Biradar and Rayburn, 1993; Rayburn *et al.*, 1993). That the near isolines of Chisholm also had nuclear DNA contents that were similar to the recurrent parent also indicated a stable nuclear DNA content inheritance. However, since Atlas and Chisholm had similar genome sizes, one cannot infer how backcrossing resulted in a genome size similar to the recurrent parent.

That the near isolines had genome sizes similar to the recurrent parents is important for additional reasons. In recent years, questions as to the validity and reproducibility of intraspecific nuclear DNA content variation in plants have been raised (Greilhuber and Ebert, 1994; Bennett and Leitch, 1995; Greilhuber and Obermayer, 1997). Skepticism as to whether small intraspecific DNA content can be documented in plants now exists. The results of this study clearly demonstrate that if experiments are correctly designed small differences can be observed in the nuclear DNA content of wheat. In addition, the variation that is observed in this study does have biological significance. If nuclear DNA content is stably inherited in wheat, one would have expected that the near isolines should have a nuclear DNA content similar to the recurrent parent. The three near isolines in the Century background all had nuclear DNA contents similar to Century with no significant difference between themselves or with Century. In order to further test the reproducibility of the technique, four of the near isolines were reanalysed in a separate experiment. The results obtained were the same as when all nine lines were analysed. The Chisholm near isolines grouped together in one grouping while the Century near isolines grouped into a second group. All nuclear DNA analyses were run blind. The fact that the near isolines grouped with their recurrent parent, without exception, testifies to the reliability and the reproducibility of the flow cytometric techniques used in this study.

In conclusion, flow cytometric analysis was found to be accurate and reliable in detecting nuclear DNA variation of < 1% in wheat. Nuclear DNA variation in the near isolines studied indicates that the variation was associated with pedigree and was not associated with aluminium response. The breeding scheme used to develop the hard red winter wheat near isolines did not induce any unusual nuclear DNA content variation.

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